Differential expression of hydroxyurea transporters in normal and polycythemia vera hematopoietic stem and progenitor cell subpopulations

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Abstract: Polycythemia vera (PV) is a myeloproliferative neoplasm marked by hyperproliferation of the myeloid lineages and the presence of an activating JAK2 mutation. Hydroxyurea (HU) is a standard treatment for high-risk patients with PV. Because disease-driving mechanisms are thought to arise in PV stem cells, effective treatments should target primarily the stem cell compartment. We tested for the antiproliferative effect of patient treatment with HU in fluorescence-activated cell sorting-isolated hematopoietic stem/multipotent progenitor cells (HSC/MPPs) and more committed erythroid progenitors (common myeloid/megakaryocyte-erythrocyte progenitors [CMP/MEPs]) in PV using RNA-sequencing and gene set enrichment analysis. HU treatment led to significant downregulation of gene sets associated with cell proliferation in PV HSCs/MPPs, but not in PV CMP/MEPs. To explore the mechanism underlying this finding, we assessed for expression of solute carrier membrane transporters, which mediate transmembrane movement of drugs such as HU into target cells. The active HU uptake transporter OCTN1 was upregulated in HSC/MPPs compared with CMP/MEPs of untreated patients with PV, and the HU diffusion facilitator urea transporter B (UTB) was downregulated in HSC/MPPs compared with CMP/MEPs in all patient and control groups tested. These findings indicate a higher accumulation of HU within PV HSC/MPPs compared with PV CMP/MEPs and provide an explanation for the differential effects of HU in HSC/MPPs and CMP/MEPs of patients with PV. In general, the findings highlight the importance of transporter expression in linking therapeutics with human disease.

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Differential expression of hydroxyurea transporters in normal and polycythemia vera hematopoietic stem and progenitor cell subpopulations

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Polycythemia vera (PV) is a myeloproliferative neoplasm marked by hyperproliferation of the myeloid lineages and the presence of an activating *JAK2* mutation. Hydroxyurea (HU) is a standard treatment for high-risk patients with PV. Because disease-driving mechanisms are thought to arise in PV stem cells, effective treatments should target primarily the stem cell compartment. We tested for the antiproliferative effect of patient treatment with HU in fluorescence-activated cell sorting-isolated hematopoietic stem/multipotent progenitor cells (HSC/MPPs) and more committed erythroid progenitors (common myeloid/megakaryocyte−erythrocyte progenitors [CMP/MEPs]) in PV using RNA-sequencing and gene set enrichment analysis. HU treatment led to significant downregulation of gene sets associated with cell proliferation in PV HSCs/MPPs, but not in PV CMP/MEPs. To explore the mechanism underlying this finding, we assessed for expression of solute carrier membrane transporters, which mediate transmembrane movement of drugs such as HU into target cells. The active HU uptake transporter OCTN1 was upregulated in HSC/MPPs compared with CMP/MEPs of untreated patients with PV, and the HU diffusion facilitator urea transporter B (UTB) was downregulated in HSC/MPPs compared with CMP/MEPs in all patient and control groups tested. These findings indicate a higher accumulation of HU within PV HSC/MPPs compared with PV CMP/MEPs and provide an explanation for the differential effects of HU in HSC/MPPs and CMP/MEPs of patients with PV. In general, the findings highlight the importance of transporter expression in linking therapeutics with human disease.

Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by hyperproliferation of the erythroid, megakaryocytic, and granulocytic lineages and the presence of an activating mutation in *JAK2* [1−6]. Clinically, PV is marked by erythrocytosis and a concomitant increase in the risk of thrombotic events [1]. Disease-driving pathogenic changes are thought to arise in stem cells that give rise to the diseased cell clone [7−9]. In healthy individuals, hematopoietic stem/multipotent progenitor cells (HSC/MPPs) can differentiate into committed progenitor cells, including common myeloid/megakaryocyte-erythrocyte progenitors (CMP/MEPs) [10−12]. When the HSC differentiation process is changed, for example, upon genetic or epigenetic alterations in HSCs, abnormal stem cell subpopulations may form, leading to clonal hematopoiesis and the onset of myeloid disease [13,14].

Treatment of PV is aimed at the prevention of thrombotic events [1], and typical frontline management includes

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a combination of low-dose aspirin and phlebotomy to decrease hematocrit to <45% [15,16]. For high-risk patients with PV, cytoreductive therapy with hydroxyurea (HU) is a standard treatment against cardiovascular complications [17]. HU acts as a potent ribonucleotide reductase inhibitor and inhibits cell proliferation by diminishing intracellular ribonucleotide pools [18,19]. This antiproliferative effect of HU is dependent on active uptake of HU into the hyperproliferating PV hematopoietic stem and progenitor cells. The transmembrane movement of HU is mediated by solute carrier membrane transporters, such as OCTN1 (organic cation transporter, novel, type1, *SLC22A4*), OCTN2 (organic cation transporter, novel, type2, *SLC22A5*), UTA (urea transporter A, *SLC14A2*), UTB (urea transporter B, *SLC14A1*), OATP1A2 (organic anion transporting polypeptide 1A2, *SLCO1A2*), OATP1B1 (organic anion-transporting polypeptide 1B1, *SLCO1B1*), and OATP1B3 (organic anion-transporting polypeptide 1B3, *SLCO1B3*) [20]. Systematic research on these and other solute carrier membrane transporters is deemed essential to link therapeutics with human disease [21]. Furthermore, HU transporter expression has not been assessed in disease-initiating HSC/MPPs and more committed progenitor cells in patients with PV. We thus performed RNA-sequencing experiments with quantitative polymerase chain reaction (qPCR) validation in HSC/MPPs and CMP/MEPs of untreated and HU-treated chronic and progressed patients with PV, as well as controls, to assess potential differential effects of HU and HU transporter expression in PV hematopoietic stem and progenitor cell subpopulations.

Methods

Human hematopoietic stem/progenitor cell samples

Fresh human peripheral blood samples were collected from untreated patients with PV, patients with PV treated with HU, and controls with phlebotomy-requiring hemochromatosis during clinical routine phlebotomy appointments (Department of Medical Oncology and Hematology, University Hospital Zurich, Zurich, Switzerland) (Table 1). All samples were collected with informed consent, and the experiments were approved by the responsible local ethics committee (Kanton Zurich, Switzerland).

To obtain the required cell numbers for the different hematopoietic stem and progenitor cell subpopulations, 0.4–3.6 L of blood had to be collected from individual patients in consecutive settings within periods of a few months. Because such volumes cannot be retrieved from individual healthy donors, we recruited hemochromatosis patients that are physically healthy and receive regular phlebotomies as control subjects.

Cell preparation, flow-cytometric analysis, cell sorting, and sample preparation for RNA-sequencing

Human hematopoietic stem cell-enriched subfractions and erythroid progenitors were isolated using fluorescence-activated cell sorting (FACS) as previously described [22] and depicted in Figure 1. In brief, mononuclear cells were isolated using Ficoll gradient centrifugation (VWR, Dietikon, Switzerland). CD34+ hematopoietic stem and progenitor cells were then enriched from mononuclear cells using immunomagnetic beads according to the manufacturer's instructions (CD34 MicroBead Kit; Miltenyi Biotec, Bergisch Gladbach, Germany) and viably frozen. After thawing, HSC/MPPs and CMP/MEPs were isolated by FACS from CD34+-enriched cells using anti-hCD34, anti-hCD38, anti-hCD123, anti-hCD45RA, and 12 antibodies against lineage markers (anti-hCD2, anti-hCD3, anti-hCD4, anti-hCD7, anti-hCD8, anti-hCD10, anti-hCD11b, anti-hCD14, anti-hCD19, anti-hCD20, anti-hCD56, anti-hCD235a). A complete list of the antibodies used is provided in Supplementary Table E1 (online only, available at www.exphem.org). Up to 10,000 HSC/MPPs and CMP/MEPs were sorted into RNeasy lysis buffer (Qiagen, Hilden, Germany) with β-mercaptoethanol for subsequent RNA-sequencing experiments.

RNA isolation and sequencing

Total RNA was isolated and purified according to the manufacturer's instructions using the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany). RNA sequencing was performed as delineated in Picelli et al. [23] using the NovaSeq sequencing platform (Illumina, San Diego, CA).

RNA-sequencing data analysis

Adapters and low-quality tails were trimmed from reads before the reads were mapped to the transcriptome. STAR aligner (Version 2.6.1.c) [24] was employed to align the RNA-sequencing data to Ensembl Release 91 reference genome build GRCh38.p10. Gene expression values were determined using featureCounts from the Bioconductor package Rsubread (Version 1.32.4) [25]. Differential gene expression was calculated with DESeq2 package (Version 1.22.2) [26].

Mutational analysis from RNA-sequencing data

We followed the GATK *Best Practices Workflow* [27]. Mapped RNA reads were duplicates marked, split, and base quality recalibrated. Variants were called by HaplotypeCaller from GATK (Version 4.0.8.1) [27] and annotated with Ensembl VEP [28]. We tested for the *JAK2* V617F mutation and estimated its allele burden.

Variant allele frequency determination in granulocytes using droplet digital PCR

Granulocytes were collected from patients with PV using centrifugation and treatment with 150 mM NH_4Cl/10 mM $KHCO_3$/0.1 mM Na_2EDTA to remove contaminating erythrocytes. Genomic DNA was extracted with the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Droplet digital PCR (ddPCR) for *JAK2*-V617F variant allele frequency determination was performed with the Bio-Rad QX200 ddPCR system and the assay dHsaMDV2010061 (BioRad, Hercules, CA) using the manufacturer's protocol.

Gene ontology enrichment analysis

Gene sets for gene set enrichment analysis were retrieved from the Gene Ontology Consortium database (www.geneontology.org) on May 26, 2019. Ranked lists were built from

Table 1. Characteristics of patients with PV and controls

Group	Subgroups combined	Patient ID	Age	Sex	Diagnosis	No. of blood samples collected	*JAK2* V617F allele burden in HSC/MPPs/ CMP/MEPs based on RNA-seq data	*JAK2* V617F allele burden in granulocytes based on ddPCR	Therapy
PV	PVchronUT	PV5*	59	M	PV	2	20%/70%	43%	ASA
PV	PVchronUT	PV6	53	M	PV	5	70%/100%	93%	ASA
PV	PVchronUT	PV8	68	F	PV	1	0%/80%		ASA, BP, statin
PV	PVchronUT	PV13	55	M	PV	5	100%/100%	78%	ASA, BP
PV	PVchronUT	PV14	44	F	PV	1	n.a./100%	97%	ASA, anti-depressant
PV	PVchronUT	PV16	50	M	PV	6	0%/50%	43%	ASA
PV	PVprogUT	PV17	64	M	Post-PV MF	1	80%/100%		BP, anti-uric, prednisone
PV	PVprogUT	PV18	72	F	PV transforming into MF	2	100%/100%	98%	ASA, BP, anti-uric
PV	PVchronHU	PV2	65	M	PV	8	0%/0% (GMPs: 20%)		HU, ASA, BP, statin
PV	PVchronHU	PV3	77	M	PV	9	80%/50%	95%	HU, ASA, BP
PV	PVchronHU	PV4	57	M	PV	7	0%/n.a.	4%	HU, ASA, BP
PV	PVchronHU	PV5	60	M	PV	5	45%/25%	43%	HU, ASA
PV	PVprogHU	PV10	65	M	PV transforming into AML	5	75%/70%		HU, ASA
PV	PVprogHU	PV11	61	M	Post-PV MF	1	60%/100%	99%	HU, ASA
PV	PVprogHU	PV15	82	M	Post-PV MF	1	100%/50%		HU, phenprocoumon , anti-uric
Control	Control	CON1	71	M	Hemochromatosis	8	0%/0%		BP
Control	Control	CON2	42	M	Hemochromatosis	3	0%/0%		None
Control	Control	CON2	66	M	Hemochromatosis	3	0%/0%		ASA, BP, anti-depressant
Control	Control	CON2	61	F	Hemochromatosis	1	0%/0%		Inhal
Control	Control	CON2	40	M	Hemochromatosis	2	0%/0%		none
Control	Control	CON3	54	M	Hemochromatosis	9	0%/0%		ASA, anti-diabetic
Control	Control	CON4	45	M	Hemochromatosis	3	0%/0%		Thyroxine
Control	Control	CON4	60	F	Hemochromatosis	3	0%/0%		BP, HRT
Control	Control	CON5	63	M	Hemochromatosis	8	0%/0%		Statin

AML=Acute myeloid leukemia; *anti-uric*=uric acid-lowering medication; *ASA*=acetylsalicylic acid; *BP*=blood pressure medication; *F*=female; *GMP*=granulocyte−macrophage progenitors; *HRT*=hormone replacement therapy; *HU*=under treatment with hydroxyurea; *Inhal*=inhalative medication; *M*=male; *MF*=myelofibrosis; *n.a.*=not available; *UT*=untreated.

*Patient previously on HU (stopped because of side effects).

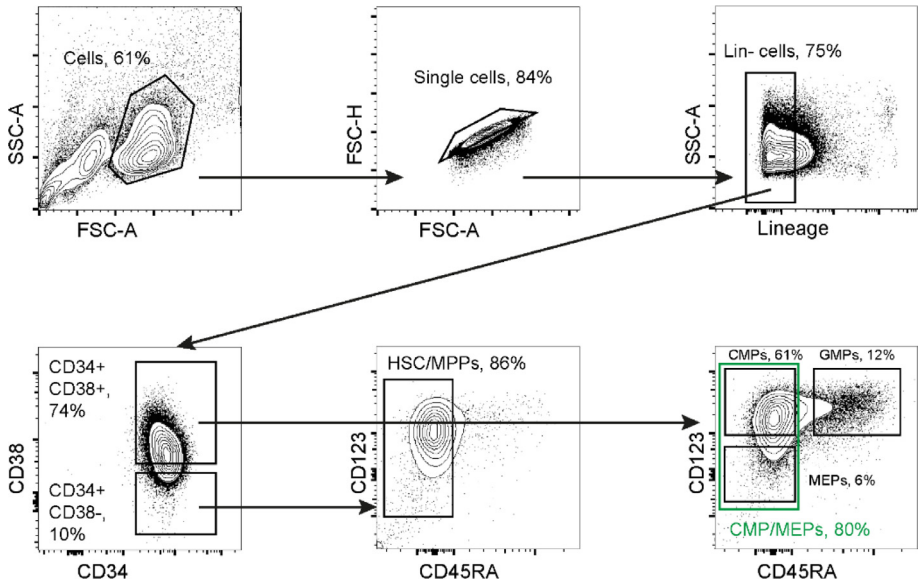

Figure 1. FACS isolation of hematopoietic stem and progenitor cell subpopulations from patients with PV and controls. Intact cells were selected on the basis of forward scatter/side scatter (FSC/SSC). Doublets were excluded using area and height of FSC (FSC-A and FSC-H). Lymphatic and differentiated cells were excluded using a combination of 12 lineage antibodies. The stem and progenitor cell markers CD34 and CD38 were employed to select stem cell-enriched cells ($Lin^-CD34^+CD38^-$) and myeloid progenitor cells ($Lin^-CD34^+CD38^+$). Stem cell-enriched cells were further purified to hematopoietic stem and multipotent progenitor cells (HSC/MPPs) using CD45RA. Myeloid progenitor cells were subdivided into common myeloid progenitors (CMPs), megakaryocyte–erythrocyte progenitors (MEPs), and granulocyte–macrophage progenitors (GMPs) using the markers CD123 and CD45RA. HSC/MPPs and CMP/MEPs were sorted in uniform cell numbers for downstream analyses.

the normalized and filtered transcriptome data using $\log_2$ (fold change) as ranking criterion. Only RNAs expressed in at least half of the replicates in both comparison groups were considered. Gene set enrichment analysis was run on pre-ranked lists using Version 4.1.0 of the software (http://www.broadinstitute.org/gsea) with default settings [29]. Significance was defined stringently by a false discovery rate (FDR) <0.05.

Quantitative polymerase chain reaction

Isolated mRNA was reverse transcribed using the SuperScript IV VILO Master Mix with ezDNase enzyme (SuperScript IV Vilo Master Mix with ezDNase Enzyme, ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions. Quantitative analysis of cDNA was performed employing Taqman probes and master mix (TaqMan Fast Advanced Master Mix, ThermoFisher Scientific). Individual probes included Hs00998199_m1 (UTB, SLC14A1), Hs00268200_m1 (OCTN1, SLC22A4), and Hs99999903_m1 (ACTB) (ThermoFisher Scientific). ACTB was used as housekeeping control gene. Expression values were derived using a ΔCt approach.

Additional statistical analyses

Statistical significance of RNA expression between different patient and control groups and between HSC/MPPs and CMP/MEPs was determined by two-way analysis of variance and fitted mixed model analyses, correcting for multiple testing using the Tukey method, and by multiple *t* tests using the Holm-Sidak method for correction for multiple testing. Regression analyses were performed based on ordinary least-squares estimation. Principal component analysis was performed on FPKM (fragments per kilobase of transcript per million mapped reads) expression values using the prcomp function in R.

Data availability

The transcriptomics data have been deposited to the gene expression omnibus (GEO) repository under accession number GSE145802.

Results

Treatment with HU has differential effects on HSC/MPPs compared with CMP/MEPs of patients with PV

To study the molecular effects of treatment with HU in undifferentiated stem/multipotent progenitor cells and more committed erythroid progenitors of patients with PV, we determined the transcriptomic profiles of HSC/MPPs and CMP/MEPs isolated from 99 blood samples of 8 untreated patients with PV, 7 HU-treated patients with PV, and 9 controls. Consecutive samples from the same individual patients had to be pooled to ensure adequate cell numbers in the different FACS-isolated hematopoietic stem and progenitor cell subpopulations. In two instances, blood samples from different control individuals had to be pooled to maintain constant input cell numbers (10,000 cells). An average of 14,272 RNAs per individual sample were identified.

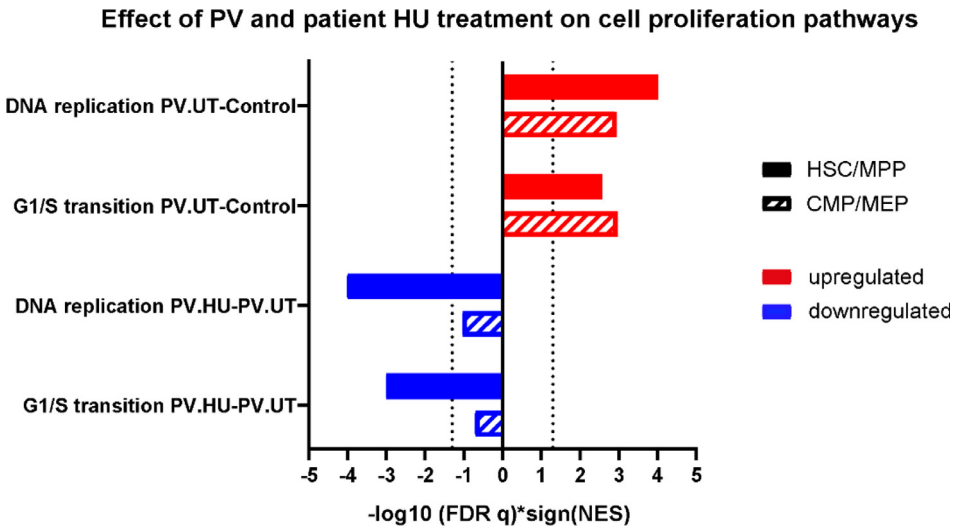

Figure 2. Effect of PV and patient treatment with HU on cell proliferation gene ontologies in different PV hematopoietic stem and progenitor cell subpopulations. Upregulated gene ontologies are marked in *red*, and downregulated gene ontologies are marked in *blue*. Significance (FDR = 0.05) is marked by *dotted lines*. *PV.UT*=PV patients without HU treatment; *PV.HU*=PV patients treated with HU; *NES*=normalized enrichment score.

A detailed overview of patient characteristics and sample distributions is provided in Table 1. The majority of patients with PV and controls studied were male (80% of PV patients and 78% of controls). The average ages of patients with PV and controls at times of sample collection were similar (62 years for PV patients and 56 years for controls). If patients with PV were diagnosed with myelofibrosis or acute myeloid leukemia within 1 year after sample collection and were in a transition state at the time of sampling, they were referred to as progressed PV (PVprog) as opposed to chronic PV (PVchron). The overall gene expression profile of progressed and more severe patients with PV was similar to that of chronic PV patients (Supplementary Figure E1, online only, available at www.exphem.org). At the time of sample collection, 7 of the 15 PV patients studied received HU treatment. Thereby, only patients that clinically responded to HU were included in the study. All patients with PV (but no controls) carried the *JAK2* V617F mutation. Allele burdens varied between 4% and 100%. Different allele burdens were observed in HSC/MPPs, CMP/MEPs, and granulocytes, with consistently higher allele burdens seen in more differentiated CMP/MEPs compared with HSC/MPPs in untreated patients with PV, but not in HU-treated patients (Table 1).

To examine the impact of polycythemia vera and patient treatment with HU on cell proliferation pathways, gene set enrichment analysis was performed for the gene ontologies of DNA replication and G1/S transition of the mitotic cell cycle. Significant upregulation of these pathways was seen both in HSC/MPPs and CMP/MEPs of untreated patients with PV compared with controls (Figure 2), suggesting increased hematopoietic stem and progenitor cell proliferation in untreated patients with PV. Patient treatment with HU resulted in strong and highly significant downregulation of the DNA replication and G1/S transition of the mitotic cell cycle pathways in PV HSC/MPPs. In contrast and using a stringent cutoff of FDR = 0.05, in CMP/MEPs no significant downregulation of the DNA replication and G1/S transition of the mitotic cell cycle gene ontologies was observed (Figure 2). In summary, patient treatment with HU had a strong negative effect on cell proliferation pathways in undifferentiated HSC/MPPs, but not in more committed erythroid progenitors (CMP/MEPs).

Expression of solute carrier membrane transporters in HSC/MPPs and CMP/MEPs of patients with PV

Possible reasons for the differential effect of patient HU treatment in PV HSC/MPPs and PV CMP/MEPs include the differential expression of solute carrier membrane transporters. We thus tested for the expression of solute carrier membrane transporters in HSC/MPPs and CMP/MEPs of patients with PV and controls (Figure 3). Among the 57 solute carrier membrane transporters studied, 29 were expressed in PV and normal hematopoietic stem and progenitor cell samples. Most of these solute carrier membrane transporters were equally expressed in PV and normal hematopoietic stem and progenitor cell subpopulations. Exceptions included the monocarboxylate transporter MCT4, the peptide transporter PEPT2, and the organic cation and HU transporter OCTN1, with PEPT2 and OCTN1 being more highly expressed in PV HSC/MPPs and

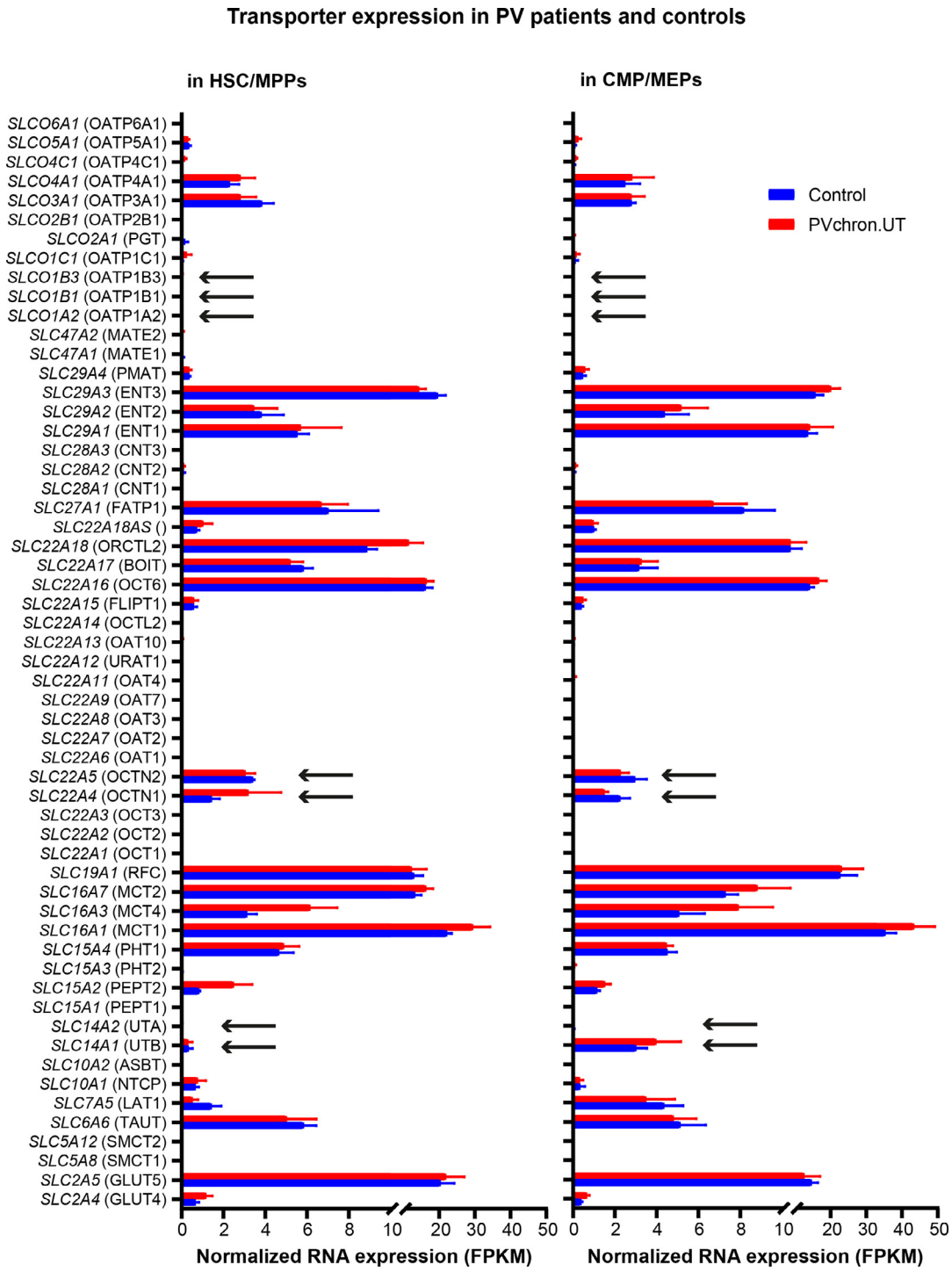

Figure 3. Expression of solute carrier membrane transporters based on RNA-sequencing data in PV and control hematopoietic stem and progenitor cell subpopulations. Denoted are the solute carrier genes encoding individual membrane transporters (in parentheses). *Arrows* mark HU transporters. Detailed information on all genes listed is provided in Supplementary Table E2 (online only, available at www.exphem.org) and under http://slc.bioparadigms.org/.

MCT4 being more highly expressed in both PV HSC/MPPs and PV CMP/MEPs compared with controls (Figure 3). Among the known HU transporters, OCTN1, OCTN2, UTB, and, to a reduced extent, UTA and OATP1B3 were expressed in hematopoietic stem and progenitor cells of patients with PV and controls. The HU transporters OATP1A2 and OATP1B1 were expressed neither in PV nor in control hematopoietic stem and progenitor cells (Figures 3 and 4). Detailed explanations of all solute carrier membrane transporters

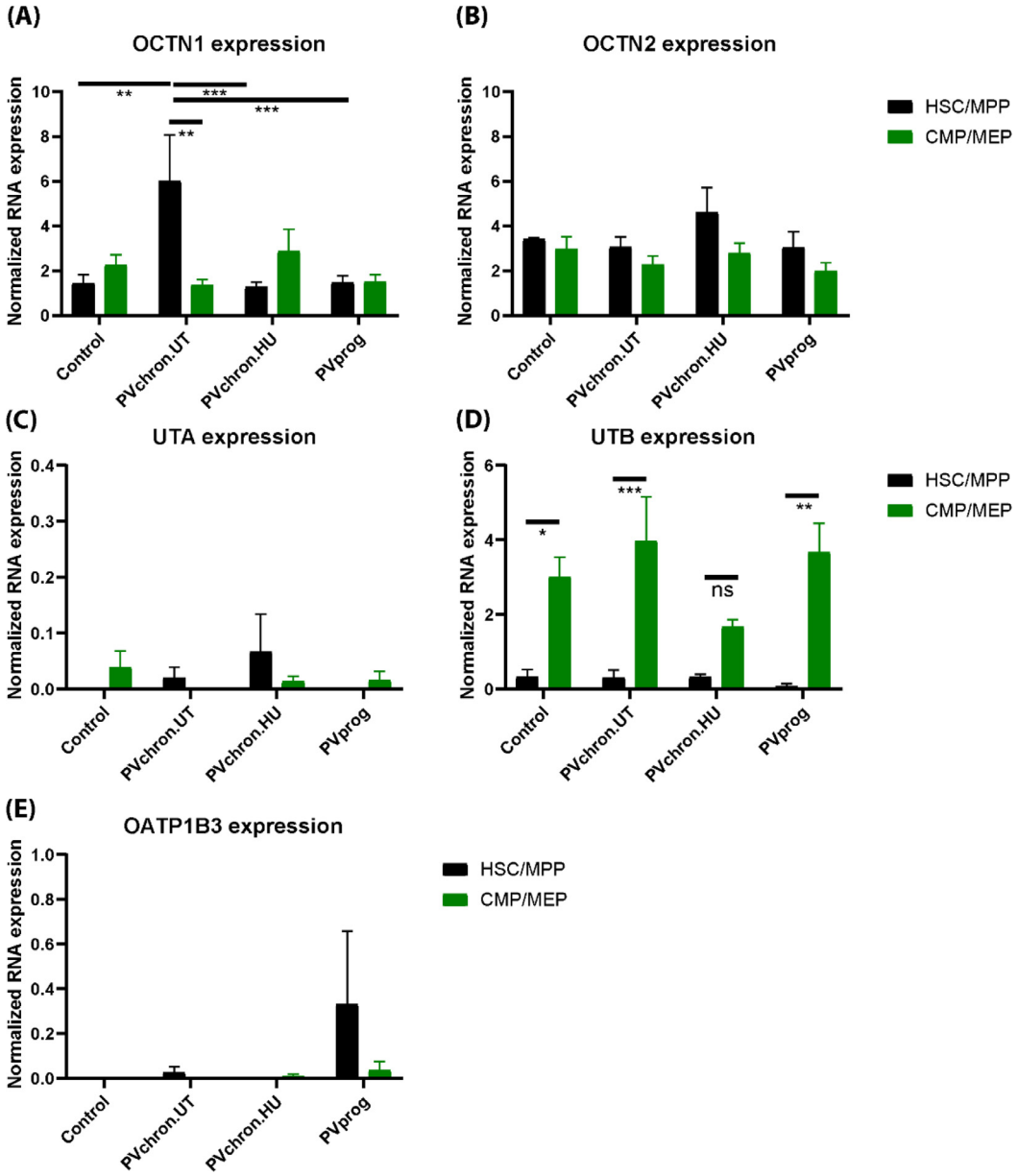

Figure 4. Expression of HU transporters based on RNA-sequencing data in different PV patient/control groups and hematopoietic stem and progenitor cell subpopulations. As OCTN1 correlated with hematocrit, its expression was examined in PVchron.UT patients with a hematocrit ≥10% above the normal lower limit. Expression of the other transporters was examined independently of the hematocrit in all patients. Expression changes in HSC/MPPs and CMP/MEPs of controls, untreated patients with chronic PV, and HU-treated patients with chronic PV were validated by qPCR (see Supplementary Figure E2). *Adjusted $p < 0.05$. **Adjusted $p < 0.01$. ***Adjusted $p < 0.001$. Error bars represent standard errors.

studied are provided in Supplementary Table E2 (online only, available at www.exphem.org) and at http://slc.bioparadigms.org/.

Differential expression of the HU transporter OCTN1 and HU diffusion facilitator UTB in HSC/MPPs and CMP/MEPs of patients with PV

We next compared HU transporter expression in the hematopoietic stem and progenitor cell subpopulations of controls and different PV patient groups (Figure 4). Differential expression was observed for the active HU transporter OCTN1 but not for OCTN2 and UTA (Figure 4A–C). For the HU diffusion facilitator UTB, significant expression changes were observed between different hematopoietic stem and progenitor cell subpopulations (Figure 4D), whereas no significant expression differences were seen for the active HU transporter OATP1B3 (Figure 4E). OCTN1 expression

correlated with hematocrit levels (R^2=0.537) [30], and we identified a significant increase in OCTN1 expression in HSC/MPPs of untreated patients with chronic PV with hematocrits ≥10% above the normal lower limit compared with CMP/MEPs in the same patient group and also compared with HSC/MPPs of HU-treated patients with chronic PV, patients with progressed PV, and controls (Figure 4A). In contrast, UTB was preferentially expressed in CMP/MEPs compared with HSC/MPPs in all patient and control groups tested (Figure 4D). These OCTN1 and UTB expression patterns in HSC/MPPs and CMP/MEPs of patients with PV and controls were validated using an orthogonal methodology (qPCR) (Supplementary Figure E2, online only, available at www.exphem.org).

All patients with PV analyzed carried the *JAK2* V617F mutation (Table 1), yet expression of OCTN1 and UTB did not correlate with *JAK2* V617F allele burden (R^2 = 0.083 and 0.098, respectively). Upregulation of OCTN1 in HSC/MPPs of untreated patients with PV is thus a *JAK2* V617F allele burden-independent disease manifestation. Whereas OCTN1 is an active HU uptake transporter, UTB is a mediator of bidirectional facilitated HU diffusion [20,31]. Differential expression of OCTN1 and UTB in HSC/MPPs and CMP/MEPs of patients with PV can explain the differential effects of patient treatment with HU in the two hematopoietic stem and progenitor cell subpopulations.

Discussion

This study identified differential expression of the HU transporters OCTN1 and UTB in HSC/MPPs and CMP/MEPs of patients with PV as a possible explanation for the observed differential effects of HU on cell proliferation pathways in different PV hematopoietic stem and progenitor cell subpopulations.

Examining the effect of HU in different hematopoietic stem and progenitor cell subpopulations in PV patients and controls, we observed a strong inhibitory effect of HU on activated cell proliferation pathways in PV HSC/MPPs but not in PV CMP/MEPs. We next assessed for the expression of 57 different solute carrier membrane transporters [21] in HSC/MPPs and CMP/MEPs of untreated and HU-treated patients with PV as well as controls and identified upregulation of the HU uptake transporter OCTN1 in PV HSC/MPPs compared with PV CMP/MEPs and controls. OCTN1 expression was previously reported in normal myeloid cells of the erythroid lineage [32] and found to correlate with proliferation and the efficacy of HU treatment in vitro [30,31]. PV is an in vivo proliferation model, and in line with the in vitro observations, we saw the highest expression of OCTN1 in HSC/MPPs of untreated patients with PV and, thus, in the group that showed activation of proliferation pathways and the strongest inhibitory effect of HU. OCTN1 is a pH-dependent transporter [33]. Although in murine hematopoietic stem and progenitor cells, intracellular pH was found to be similar to the pH of blood plasma, increased intracellular pH values were observed with increased proliferation activity of hematopoietic stem and progenitor cells [34]. These previous observations underscore the importance of high OCTN1 expression in highly proliferating HSC/MPPs of untreated patients with PV (Figure 4A).

In addition to upregulated OCTN1, we identified downregulation of UTB expression in HSC/MPPs compared with CMP/MEPs in patients with PV and controls (Figure 4D). Because UTB transports HU bidirectionally via facilitated diffusion [20], its high expression prevents intracellular accumulation of HU. Facilitated diffusion of HU depends on the electrochemical gradient across the cell membrane, which was measured at −12mV in red blood cells [35]. This electrochemical gradient is small compared with those of other cell types (e.g., −70 to −80mV in neurons). Assuming a similarly low electrochemical gradient in hematopoietic stem and progenitor cells, a weak inward flux of hydroxyurea via UTB is expected at low intracellular HU concentrations, whereas high intracellular HU concentrations favor the outward flux of hydroxyurea through UTB.

mRNA does not always predict solute carrier protein levels. For soluble proteins, we observed positive mRNA−protein correlations for 70% of all identified proteins [22,36]. This overall positive correlation of protein and RNA expression in hematopoietic stem and progenitor cells of patients with PV and controls suggests that RNA levels are a reasonable approximate substitute for protein expression. This study is a first step in determining possible drug uptake mechanisms for hydroxyurea in PV HSC/MPPs.

Our study suggests that OCTN1 expression may predict patients' response to hydroxyurea. However, we do not know the response of the untreated patients with PV to HU, and we thus cannot exclude the possibility of compensatory mechanisms that may reduce the expression of OCTN1 in HU-treated patients without impairing the proliferation response. Prospective studies correlating the expression of OCTN1 with patients' response to HU are warranted. Furthermore, the hematopoietic stem and progenitor cells analyzed in the study were isolated from the peripheral blood. It remains to be determined whether similar expression changes exist in bone marrow hematopoietic stem and progenitor cells.

In summary, high OCTN1 expression and low UTB expression favor intracellular accumulation of HU in PV HSC/MPPs, whereas low OCTN1 expression favors low intracellular HU levels in PV CMP/MEPs. Hence, our

findings of differential expression of OCTN1 and UTB in PV HSC/MPPs and PV CMP/MEPs could well explain the differential effects of HU on cell proliferation pathways in these two PV hematopoietic stem and progenitor cell subpopulations. Overall, our results highlight the importance of transporter expression in linking therapeutics with human disease.

Conflict of interest disclosure

The authors declare no competing financial interests.

Acknowledgments

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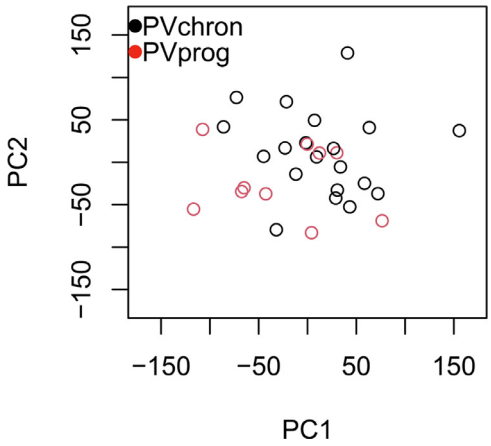
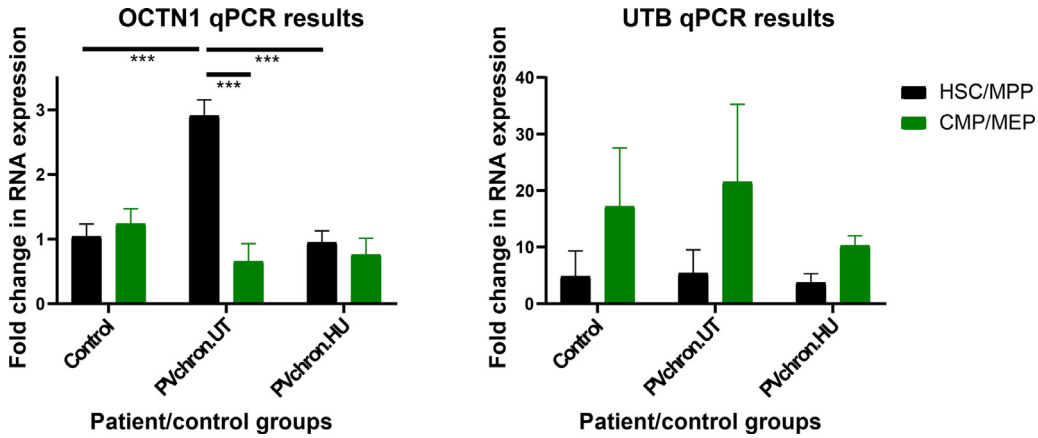


Supplementary Figure 1. Principal component analysis in all PV samples analyzed. Samples from chronic (PVchron) and more severe and progressed (PVprog) PV patients demonstrated comparable overall gene expression profiles with no apparent outliers observed.



Supplementary Figure 2. qPCR validation of OCTN1 and UTB expression in HSC/MPPs and CMP/MEPs of untreated and HU-treated chronic PV patients and controls. Shown are fold changes of RNA expression where fold change was defined as 2^Delta(DeltaCt) and Delta(DeltaCt) = (Ct(beta-actin house keeping gene in a specific sample)-Ct(test gene in same specific sample)) - Mean(Ct(beta-actin house keeping gene in Control.HSC/MPP)-Ct(test gene in Control.HSC/MPP)). n=9, error bars represent standard errors. ***Adjusted $p < 0.001$. *Abbreviations*: OCTN1= organic cation transporter, novel, type1; UTB= urea transporter B; PVchron. UT = patients with chronic PV without cytoreductive therapy; PVchron.HU = chronic PV patients with HU treatment.

Supplementary Table 1.

Antibody	Clone	Provider	Order number
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD2	S5.5	ThermoFisherScientific-Invitrogen	CD0206
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD3	7D6	ThermoFisherScientific-Invitrogen	MHCD03065
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD4	S3.5	ThermoFisherScientific-Invitrogen	MHCD0406
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD7	CD7-6B7	ThermoFisherScientific-Invitrogen	MHCD0706
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD8	3B5	ThermoFisherScientific-Invitrogen	MHCD0806
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD14	TuK4	ThermoFisherScientific-Invitrogen	MHCD1406
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD19	SJ25-C1	ThermoFisherScientific-Invitrogen	MHCD1906
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD56	MEM-188	ThermoFisherScientific-Invitrogen	MHCD5606
Phycoerythrin (PE)-Cy5 anti-hCD10	HI10a	BioLegend	312206
Phycoerythrin (PE)-Cy5 anti-hCD11b	ICRF44	BioLegend	301308
Phycoerythrin (PE)-Cy5 anti-hCD20	2H7	BioLegend	302308
Phycoerythrin (PE)-Cy5 anti-hCD235a	GA-R2	BD Biosciences	559944
PE-Cy7-conjugated anti-hCD34	8G12	BD Biosciences	348811
FITC-conjugated anti-hCD38	HIT2	BD Biosciences	555459
APC-conjugated anti-hCD123	6H6	ThermoFisherScientific-Invitrogen	17-1239-42
APC780-conjugated anti-hCD45RA	HI100	ThermoFisher Scientific-Invitrogen	47-0458-41

Supplementary Table 2.

Suppl Table 2

Gene name	Full gene name	Protein name	Full protein name	Function (as defined by UniProtKB/Swiss-Prot)
SLC2A4	Solute Carrier Family 2 Member 4	GLUT4	Glucose Transporter Type 4	Insulin-regulated facilitative glucose transporter
SLC2A5	Solute Carrier Family 2 Member 5	GLUT5	Glucose Transporter Type 5	Fructose transporter
SLC5A8	Solute Carrier Family 5 Member 8	SMCT1	Sodium-Coupled Monocarboxylate Transporter 1	Electrogenic sodium-coupled solute transporter for transport of monocarboxylates
SLC5A12	Solute Carrier Family 5 Member 12	SMCT2	Sodium-Coupled Monocarboxylate Transporter 2	Electroneutral sodium-coupled solute transporter for transport of monocarboxylates
SLC6A6	Solute Carrier Family 6 Member 6	TAUT	Taurine Transporter	Sodium-dependent taurine and beta-alanine transporter
SLC7A5	Solute Carrier Family 7 Member 5	LAT1	L-Type Amino Acid Transporter 1	Sodium-independent transporter for large neutral amino acids
SLC10A1	Solute Carrier Family 10 Member 1	NTCP	Na+/Taurocholate Cotransporting Polypeptide	Hepatic sodium/bile-acid transporter
SLC10A2	Solute Carrier Family 10 Member 2	ASBT	Apical Sodium-Dependent Bile Acid Transporter	Sodium-dependent reabsorption of bile acids from small intestine
SLC14A1	Solute Carrier Family 14 Member 1	UTB	Urea Transporter 1	Urea channel that facilitates transmembrane urea transport down a concentration gradient
SLC14A2	Solute Carrier Family 14 Member 2	UTA	Urea Transporter 2	Specialized low-affinity vasopressin-regulated urea transporter
SLC15A1	Solute Carrier Family 15 Member 1	PEPT1	Peptide Transporter 1	Proton-coupled intake of oligopeptides of 2 to 4 amino acids
SLC15A2	Solute Carrier Family 15 Member 2	PEPT2	Peptide Transporter 2	Proton-coupled intake of oligopeptides of 2 to 4 amino acids
SLC15A3	Solute Carrier Family 15 Member 3	PHT2	Peptide/Histidine Transporter 2	Proton oligopeptide cotransporter
SLC15A4	Solute Carrier Family 15 Member 4	PHT1	Peptide/Histidine Transporter 1	Proton oligopeptide cotransporter
SLC16A1	Solute Carrier Family 16 Member 1	MCT1	Monocarboxylate Transporter 1	Proton-coupled monocarboxylate transporter
SLC16A3	Solute Carrier Family 16 Member 3	MCT4	Monocarboxylate Transporter 3	Proton-linked monocarboxylate transporter
SLC16A7	Solute Carrier Family 16 Member 7	MCT2	Monocarboxylate Transporter 2	Proton-coupled monocarboxylate transporter
SLC19A1	Solute Carrier Family 19 Member 1	RFC	Reduced Folate Carrier Protein	Transporter that mediates the import of reduced folates and a subset of cyclic dinucleotides
SLC22A1	Solute Carrier Family 22 Member 1	OCT1	Organic Cation Transporter 1	Translocates a broad array of organic cations with various structures and molecular weights
SLC22A2	Solute Carrier Family 22 Member 2	OCT2	Organic Cation Transporter 2	Mediates tubular uptake of organic compounds from circulation
SLC22A3	Solute Carrier Family 22 Member 3	OCT3	Organic Cation Transporter 3	Mediates potential-dependent transport of a variety of organic cations
SLC22A4	Solute Carrier Family 22 Member 4	OCTN1	Organic Cation/Carnitine Transporter 1	Sodium-ion dependent, low affinity carnitine transporter
SLC22A5	Solute Carrier Family 22 Member 5	OCTN2	Organic Cation/Carnitine Transporter 2	Sodium-ion dependent, high affinity carnitine transporter
SLC22A6	Solute Carrier Family 22 Member 6	OAT1	Organic Anion Transporter 1	Involved in the renal elimination of endogenous and exogenous organic anions
SLC22A7	Solute Carrier Family 22 Member 7	OAT2	Organic Anion Transporter 2	Mediates sodium-independent multispecific organic anion transport
SLC22A8	Solute Carrier Family 22 Member 8	OAT3	Organic Anion Transporter 3	Plays an important role in the excretion/detoxification of endogenous and exogenous organic anions, especially from the brain and kidney
SLC22A9	Solute Carrier Family 22 Member 9	OAT7	Organic Anion Transporter 7	Sodium-independent organic anion transporter which exhibits high specificity for sulfated conjugates of xenobiotics and steroid hormones
SLC22A11	Solute Carrier Family 22 Member 11	OAT4	Organic Anion Transporter 4	Mediates saturable uptake of estrone sulfate, dehydroepiandrosterone sulfate and related compounds

(*continued on next page*)

Supplementary Table 2 (*Continued*)

Suppl Table 2

Gene name	Full gene name	Protein name	Full protein name	Function (as defined by UniProtKB/Swiss-Prot)
SLC22A12	Solute Carrier Family 22 Member 12	URAT1	Urate Transporter 1	Required for efficient urate re-absorption in the kidney
SLC22A13	Solute Carrier Family 22 Member 13	OAT10	Organic Cation Transporter-Like 3	n.a.
SLC22A14	Solute Carrier Family 22 Member 14	OCTL2	Organic Cation Transporter-Like 4	n.a.
SLC22A15	Solute Carrier Family 22 Member 15	FLIPT1	Fly-Like Putative Transporter 1	Probably transports organic cations (By similarity)
SLC22A16	Solute Carrier Family 22 Member 16	OCT6	Organic Cation Transporter 6	Partially sodium-ion dependent, high affinity carnitine transporter
SLC22A17	Solute Carrier Family 22 Member 17	BOIT	Potent Brain Type Organic Ion Transporter	Cell surface receptor for LCN2 (24p3) that plays a key role in iron homeostasis and transport
SLC22A18	Solute Carrier Family 22 Member 18	ORCTL2	Organic Cation Transporter-Like Protein 2	May act as a transporter of organic cations based on a proton efflux antiport mechanism
SLC22A18AS	Solute Carrier Family 22 Member 18 Antisense			
SLC27A1	Solute Carrier Family 27 Member 1	FATP1	Fatty Acid Transport Protein 1	Mediates the ATP-dependent import of long-chain fatty acids (LCFA) into the cell
SLC28A1	Solute Carrier Family 28 Member 1	CNT1	Concentrative Nucleoside Transporter 1	Sodium-dependent and pyrimidine-selective transporter
SLC28A2	Solute Carrier Family 28 Member 2	CNT2	Concentrative Nucleoside Transporter 2	Sodium-dependent and purine-selective transporter
SLC28A3	Solute Carrier Family 28 Member 3	CNT3	Concentrative Nucleoside Transporter 3	Sodium-dependent, pyrimidine- and purine-selective transporter
SLC29A1	Solute Carrier Family 29 Member 1	ENT1	Equilibrative Nucleoside Transporter 1	Mediates both influx and efflux of nucleosides across the membrane (equilibrative transporter)
SLC29A2	Solute Carrier Family 29 Member 2	ENT2	Equilibrative Nucleoside Transporter 2	Mediates equilibrative transport of purine, pyrimidine nucleosides and the purine base hypoxanthine
SLC29A3	Solute Carrier Family 29 Member 3	ENT3	Equilibrative Nucleoside Transporter 3	Mediates both influx and efflux of nucleosides across the membrane (equilibrative transporter)
SLC29A4	Solute Carrier Family 29 Member 4	PMAT	Plasma Membrane Monoamine Transporter	Polyspecific organic cation transporter
SLC47A1	Solute Carrier Family 47 Member 1	MATE1	Multidrug And Toxin Extrusion Protein 1	Solute transporter for tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP), cimetidine, N-methylnicotinamide (NMN), metformin, creatinine, guanidine, procainamide, topotecan, estrone sulfate, acyclovir, ganciclovir and also the zwitterionic cephalosporin, cephalexin and cephradin
SLC47A2	Solute Carrier Family 47 Member 2	MATE2	Multidrug And Toxin Extrusion Protein 2	Solute transporter for tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP), cimetidine, N-methylnicotinamide, metformin, creatinine, guanidine, procainamide, topotecan, estrone sulfate, acyclovir, and ganciclovir
SLCO1A2	Solute Carrier Organic Anion Transporter Family Member 1A2	OATP1A2	Organic Anion-Transporting Polypeptide 1	Mediates the Na(+)-independent transport of organic anions
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1	OATP1B1	Liver-Specific Organic Anion Transporter 1	Mediates the Na(+)-independent uptake of organic anions
SLCO1B3	Solute Carrier Organic Anion Transporter Family Member 1B3	OATP1B3	Liver-Specific Organic Anion Transporter 2	Mediates the Na(+)-independent uptake of organic anions
SLCO1C1	Solute Carrier Organic Anion Transporter Family Member 1C1	OATP1C1	Organic Anion Transporter Polypeptide-Related Protein 5	Mediates the Na(+)-independent high affinity transport of organic anions

(*continued on next page*)

Supplementary Table 2 (*Continued*)

Suppl Table 2

Gene name	Full gene name	Protein name	Full protein name	Function (as defined by UniProtKB/Swiss-Prot)
SLCO2A1	Solute Carrier Organic Anion Transporter Family Member 2A1	PGT	Prostaglandin Transporter	May mediate the release of newly synthesized prostaglandins from cells
SLCO2B1	Solute Carrier Organic Anion Transporter Family Member 2B1	OATP2B1	Organic Anion Transporter Polypeptide-Related Protein 2	Mediates the Na(+)-independent transport of organic anions
SLCO3A1	Solute Carrier Organic Anion Transporter Family Member 3A1	OATP3A1	Organic Anion Transporter Polypeptide-Related Protein 3	Mediates the Na(+)-independent transport of organic anions
SLCO4A1	Solute Carrier Organic Anion Transporter Family Member 4A1	OATP4A1	Organic Anion Transporter Polypeptide-Related Protein 1	Mediates the Na(+)-independent transport of organic anions
SLCO4C1	Solute Carrier Organic Anion Transporter Family Member 4C1	OATP4C1	Organic Anion Transporter M1	Organic anion transporter, capable of transporting pharmacological substances such as digoxin, ouabain, thyroxine, methotrexate and cAMP
SLCO5A1	Solute Carrier Organic Anion Transporter Family Member 5A1	OATP5A1	Organic Anion Transporter Polypeptide-Related Protein 4	n.a.
SLCO6A1	Solute Carrier Organic Anion Transporter Family Member 6A1	OATP6A1	Testis-Specific Organic Anion Transporter	n.a.